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Geoepidemiology, clinical manifestations and outcome of primary biliary cholangitis in Greece

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ABSTRACT

Background & aims: Primary biliary cholangitis (PBC) is a disease with rising prevalence and considerable geographical variation. To describe the prevalence, spatial and time distribution, baseline characteristics, response to treatment, outcome and the validity of GLOBE score in a large cohort of Greek PBC patients as an independent validation of this score has not been done so far.

Methods: The last 16 years, 482 PBC patients (86.5% females) were evaluated and analysed retrospectively, using a prospectively collected database. Special attention was paid to the assessment of treatment response according to GLOBE score.

Results: Age at initial evaluation was 56.3 ± 13.7 years. Among 432 Thessaly residents, prevalence was 582/million (non-homogeneous distribution). Nineteen districts showed a prevalence >800/million. Symptomatic disease onset could be identified in 91 patients, with a significant peak during spring ($P = 0.03$). At diagnosis, 43.6% were asymptomatic and 16.2% cirrhotic. Male sex ($P = 0.02$), older age ($P < 0.001$), alcohol consumption ($P < 0.01$) and concomitant liver disease ($P < 0.001$) were negative prognostic factors for cirrhosis. During a median [interquartile range, range] follow-up of 5.1 (7.8, 15.7) years, 62 patients died or underwent liver transplantation. Patients with GLOBE score > 0.30 had significantly worse prognosis ($P < 0.001$) with 5-, 10-, and 15-year survival rates of 84%, 50% and 42%.

Conclusions: There is increased PBC prevalence in Thessaly with remarkable geographic clustering and seasonal variability. PBC is diagnosed at early stages although males had a more advanced disease. GLOBE score applies perfectly in Greek patients and this will likely help detecting patients that may benefit from new therapies.

1. Introduction

Primary biliary cholangitis (PBC) previously known as primary biliary cirrhosis is an autoimmune cholestatic liver disease characterized by a progressive inflammatory destruction of the small intrahepatic bile ducts with portal inflammation, progressive fibrosis, leading to cirrhosis and subsequent liver failure [1,2].

It is considered a model of autoimmune disease with specific autoantibodies, female predominance and frequent coexistence with other autoimmune conditions [1–3]. The serologic hallmark of PBC is the detection of anti-mitochondrial antibodies (AMA), directed against the E2-subunit of pyruvate dehydrogenase multi-enzyme complex located in the inner membrane of mitochondria [3,4], though several other antibodies either disease-specific or not have been detected [3,5–7]. AMA are detectable in 85–95% of patients with PBC and frequently occur long before clinical signs or symptoms appear [1–3,8].

Clinical presentation at the time of diagnosis varies from asymptomatic with normal or abnormal biochemical tests, to symptomatic, or finally to advanced liver disease. Presenting symptoms are frequently fatigue, pruritus and arthralgias in the absence of other signs of liver disease. It is now well-known, that more patients with asymptomatic PBC are being diagnosed thanks to the routine use of biochemical screening, increased medical awareness of the disease, and improvements in AMA testing [2,3,9].

The etiology of the disease remains unknown, although it is accepted that its pathogenesis has a multifactorial basis. The available evidence indicates that environmental factors may trigger PBC in genetically predisposed individuals. Genetic predisposition is supported by high concordance observed among monozygotic twins [10], high level of PBC aggregates in families [11], and significant associations with specific gene polymorphisms [12–14]. However, the concordance rate of only 63% in monozygotic twins along with the worldwide geographical heterogeneity regarding its prevalence and incidence give further support

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to the environmental impact in the development of disease clinical phenotype [10,15]. Indeed, differences noticed in PBC prevalence have been reported [16–18] and may reflect differences in exposure to environmental factors, although the determination as well the quantification of environmental influences is very difficult to be clearly defined [19]. In this context, it has been reported that environmental risk may be attributable either to infectious agents (viruses and bacteria) or xenobiotics [20,21].

Several studies have investigated the epidemiology of PBC worldwide and population-based studies, attempting to estimate the prevalence and the incidence of PBC, have introduced the concept of geoepidemiology of the disease [22], taking into account that prevalence rates among studies range from 19.1 to 402 per 1 million inhabitants and they are markedly increasing over time [23]. However, so far only one study from Crete island has described the characteristics of PBC in Greece [24]. Therefore, we conducted a retrospective analysis of prospectively collected data of a large cohort of PBC patients in order to evaluate PBC characteristics in the mainland (Central Greece, Thessaly) and to describe the spatial and time distribution of the disease in this area along with the validity of the GLOBE score as an independent validation of this score has not been done so far in PBC patients.

2. Materials and methods

2.1. Patients

A retrospective analysis of a prospectively collected database of all 482 consecutive patients with well-defined PBC, seen at the Department of Medicine, Medical School, University of Thessaly, Larissa, Greece between 2000 and 2015 was performed. Patients were followed at our outpatient clinic for a median [interquartile range (IQR), range] period of 5.1 (7.8, 15.7) years.

According to internationally accepted criteria and to our reports, PBC diagnosis was based on the presence of at least two of the following criteria: [7,25–27] (a) AMA-positivity, (b) elevated cholestatic enzymes for >6 months and (c) histological lesions of PBC.

In order to assess for possible differences in PBC distribution across Thessaly region, we have recorded the place of residence of patients. Thessaly is one of the thirteen peripheries of Greece and covers the major part of Central Greece. The prefectures of Larissa (capital city of Larissa), Magnesia (capital city of Volos), Trikala (capital city of Trikala) and Karditsa (capital city of Karditsa) and the islands of Sporades constitute Thessaly. According to the most 2 recent censuses (2001 and 2011) the population remained stable during the study period (approximately 750,000 people; 6.8% of the national population). Of note, the population of Thessaly is considered homogeneous, mainly of Caucasian origin and with a small proportion of immigrants. Residents of Thessaly were 439 out of 482 patients (91.1%) at the time point of initial evaluation.

Medical records of patients were retrieved and reviewed and data concerning patients' demographics, educational level, lifestyle, medical and family history at the period prior to PBC diagnosis, clinical and laboratory parameters at the time-point of initial evaluation, as well as outcome and treatment response during follow-up were collected. Patients with symptoms attributable to PBC, like pruritus, fatigue in the absence of other identifiable causes, abdominal pain in the right upper quadrant not attributable to another etiology, jaundice, arthralgia, sicca syndrome and major complications of end-stage liver disease (ascites, gastrointestinal bleeding due to varices, hepatic encephalopathy) were defined as symptomatic. The presence of at least one of extrahepatic autoimmune conditions was defined as “concurrence of other autoimmune diseases”. Baseline levels of alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, prothrombin time (PT), albumin, serum immunoglobulin M (IgM) were determined using standard techniques. The PBC Mayo Risk Score was calculated for all patients at diagnosis [28]. Histological staging was carried out according to Ludwig's classification [29]. For patients without an available biopsy, diagnosis of cirrhosis was based on ultrasonography (nodules in liver parenchyma, spleen > 12 cm, portal vein > 16 mm), and/or endoscopic findings of cirrhosis (varices, portal gastropathy), and/or clinical findings of decompensation. Treatment response was assessed according to the GLOBE score (≤0.30 for responders and >0.30 for non-responders) in patients treated for at least 1-year with ursodeoxycholic acid (UDCA) [30]. In brief, according to the original study by Lammers et al., a GLOBE score above the threshold of 0.30 one year after the initiation of UDCA treatment characterizes PBC patients with diminished survival compared to the general population [30]. The performance of the GLOBE score was compared to the UK-PBC risk scores [31].

2.2. Autoantibodies detection

AMA (positive titer ≥ 1/40) were initially detected by indirect immunofluorescence on 5 μm fresh frozen sections of in-house rodent multiorgan (kidney, liver and stomach) tissue substrates, as we described previously [3,7,26,32,33]. Presence of AMA was confirmed by Western blot using in-house mitochondrial subfraction of rat livers, or by enhanced performance M2 ELISA (M2 EP (MIT3) ELISA, QUANTA Lite (R), INOVA Diagnostics) [3,7,34]. PBC-specific antinuclear antibodies (ANA) namely, antibodies against nuclear pore membrane glycoprotein 210 (anti-gp210) and antibodies against nuclear sp100 protein (anti-sp100) were detected by commercial ELISAs according to the manufacturers' instructions (INOVA Diagnostics) [5,7].

2.3. Ethics approval

The study was conducted in accordance with the protocol and the principles of the Declaration of Helsinki. The protocol was reviewed and approved by the Ethics Committee of Thessaly University, Medical School.

2.4. Statistical analysis

Data were entered and analysed using SPSS and Epi-Info 2002 software. Kolmogorov-Smirnov test was used to assess the normality of the distribution of variables. Normally distributed values are expressed as mean ± standard deviation (SD), while non-normally distributed as median (IQR). Data were analysed by *t*-test, Mann-Whitney *U* test, χ^2 test (two by two with Yate's correction), Fischer's exact test, Cox regression analysis, binary logistic regression model, multiple Cox regression model, area under receiver operating characteristic curve (AUC) and Delong's test where applicable. A seasonal effect in incidence assessed by calculating individual χ^2 tests for each season comparing observed to expected values. The expected values were determined by distributing the patients equally among the 12 months of a year, assuming that no seasonal effect exists. Two-sided *P*-values <0.05 were considered as statistically significant.

3. Results

Demographic and clinical characteristics of patients at the time point of initial evaluation are shown in Table 1. There were 417 (86.5%) women (female/male ratio: 6.4:1). The age at diagnosis was 56.3 ± 13.7 years in total population, with no differences between females and males (56.3 ± 13.3 vs. 56.5 ± 15.7 years, respectively; $P = 0.883$; Supplementary Fig. 1). AMA were detected in 465/482 (96.5%) patients by using any of the three described methods (AMA, ELISA, western blot), while ANA-PBC-specific antibodies (anti-sp100 and/or anti-gp210) were found in 66/482 (13.7%); anti-sp100, $n = 48$ (10%) and anti-gp210, $n = 26$ (5.4%). Of note, AMA negative patients were significantly more frequent in ANA-PBC-specific positive patients (6/66; 9.1%) compared to those having a negative test for ANA-PBC-specific antibodies (11/416; 2.6%; $P < 0.02$). No differences were found regarding